

IN THE CLAIMS

Please amend the claims as indicated in the following listing of claims, which replaces all previous listings of claims.

1. (Previously Presented) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a DNA polymerase or reverse transcriptase, and said second enzyme is the wild type Pfu DNA polymerase comprising the amino acid sequence of SEQ ID NO. 12, except that it is mutated at an amino acid position selected from the group consisting of: Y410, T542, D543, K593, Y595, Y385, G387 and G388 and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity as compared to the wild type Pfu DNA polymerase.

2. (Cancelled)

3. (Previously Presented) The enzyme mixture of claim 1, wherein said first enzyme is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, U1Tma DNA polymerase, Tli DNA polymerase (Vent DNA polymerase), Pwo DNA polymerase, Tgo DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase having the sequence shown in SEQ ID NO. 10, PGB-D DNA polymerase (Deep Vent DNA polymerase) and DP1/DP2 DNA polymerase.

4-9. (Cancelled)

10. (Previously Presented) The enzyme mixture of claim 1, wherein said Pfu DNA polymerase mutations are one or more amino acid substitutions selected from the group consisting of: Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.
11. (Previously Presented) The enzyme mixture of claim 1, further comprising a PCR enhancing factor and/or an additive.
12. (Previously Presented) A kit comprising a first enzyme, a second enzyme, and packaging material therefor, wherein said first enzyme is a DNA polymerase or reverse transcriptase, said second enzyme is the wild type Pfu DNA polymerase comprising the amino acid sequence of SEQ ID NO. 12, except that it is mutated at an amino acid position selected from the group consisting of: Y410, T542, D543, K593, Y595, Y385, G387 and G388 and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity as compared to the wild type Pfu DNA polymerase.
13. (Cancelled)
14. (Previously Presented) The kit of claim 12, wherein said first enzyme is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, U1Tma DNA polymerase, Tli DNA polymerase (Vent DNA polymerase), Pwo DNA polymerase, Tgo DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase having the

sequence shown in SEQ ID NO. 10, PGB-D DNA polymerase (Deep Vent DNA polymerase) and DP1/DP2 DNA polymerase.

15-19. (Cancelled)

20. (Previously Presented) The kit of claim 12, further comprising one or more components selected from the group consisting of: a deoxynucleotide, a reaction buffer, a PCR enhancing factor and/or an additive, a control DNA template and a control primer.

21. (Cancelled)

22. (Previously Presented) The kit of claim 12, wherein said Pfu DNA polymerase mutations are one or more amino acid substitutions selected from the group consisting of: Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P and G388P.

23. (Withdrawn; Currently Amended) A method for DNA synthesis comprising:

(a) providing ~~an~~ said enzyme mixture of claim 1, ~~said enzyme mixture comprising a first enzyme that is a DNA polymerase or reverse transcriptase, and a second enzyme which is a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388; and~~

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

24. (Withdrawn) The method of claim 23, wherein said nucleic acid template is a DNA molecule.

25. (Cancelled)

26. (Withdrawn) The method of claim 23, wherein said first enzyme is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UITma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DPl/DP2 DNA polymerase.

27-29. (Cancelled)

30. (Withdrawn; Currently Amended) A method for DNA synthesis comprising:

(a) providing ~~an~~ said enzyme mixture of claim 1, ~~said enzyme mixture comprising a wild type Pfu DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity, and~~

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

31. (Withdrawn; Currently Amended) A method for TA cloning of DNA synthesis product comprising:

- (a) providing ~~an~~ said enzyme mixture of claim 36, ~~said enzyme mixture comprising a Taq DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity;~~
- (b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis to generate a synthesized DNA product; and
- (c) inserting said synthesized DNA product into a TA cloning vector.

32. (Cancelled)

33. (Withdrawn; Currently Amended) The method of claim 23, wherein said ~~mutant~~ Pfu DNA polymerase mutations ~~comprises~~ are one or more amino acid substitutions ~~mutations~~ selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

34. (Withdrawn) The method of claim 23, 30 or 31, wherein said reaction mixture further comprises a PCR enhancing factor and/or an additive.

35. (Withdrawn; Currently Amended) The method of claim ~~32~~ 30 or 31, wherein said ~~mutant~~ Pfu DNA polymerase mutations ~~are~~ comprises one or more amino acid substitutions ~~mutations~~ selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

36. (Previously Presented) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is Taq DNA polymerase, and said second enzyme is the wild type Pfu DNA polymerase comprising the amino acid sequence of SEQ ID NO. 12, except that it is mutated at an amino acid position selected from the group consisting of: Y410, T542, D543, K593, Y595, Y385, G387 and G388 and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity as compared to the wild type Pfu DNA polymerase.

37. (Previously Presented) The enzyme mixture of claim 36, wherein said Pfu DNA polymerase mutations are one or more amino acid substitutions selected from the group consisting of: Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

38. (Previously Presented) The enzyme mixture of claim 36, wherein said Pfu DNA polymerase is mutated at amino acid position G387.

39. (Previously Presented) The enzyme mixture of claim 36, wherein said Pfu DNA polymerase is mutated at amino acid position G387 and the resulting amino acid substitution is G387P.

40. (Previously Presented) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is KOD DNA polymerase, and said second enzyme is

the wild type Pfu DNA polymerase comprising the amino acid sequence of SEQ ID NO. 12, except that it is mutated at an amino acid position selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387 and G388 and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity as compared to the wild type Pfu DNA polymerase.

41. (Previously Presented) The enzyme mixture of claim 40, wherein said Pfu DNA polymerase mutations are one or more amino acid substitutions selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P and G388P.

42. (Previously Presented) The enzyme mixture of claim 40, wherein said Pfu DNA polymerase is mutated at amino acid position G387.

43. (Previously Presented) The enzyme mixture of claim 40, wherein said Pfu DNA polymerase is mutated at amino acid position G387 and the resulting amino acid substitution is G387P.

44. (Previously Presented) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a JDF-3 DNA polymerase having the sequence shown in SEQ ID NO. 10, and said second enzyme is the wild type Pfu DNA polymerase comprising the amino acid sequence of SEQ ID NO. 12, except that it is mutated at an amino acid position selected from the group consisting of: D405, Y410, T542, D543, K593, Y595,

Y385, G387 and G388 and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity as compared to the wild type Pfu DNA polymerase.

45. (Previously Presented) The enzyme mixture of claim 44, wherein said Pfu DNA polymerase mutations are one or more amino acid substitutions selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P and G388P.

46. (Previously Presented) The enzyme mixture of claim 44, wherein said Pfu-DNA polymerase is mutated at amino acid position G387.

47. (Previously Presented) The enzyme mixture of claim 44, wherein said Pfu DNA polymerase is mutated at amino acid position G387 and the resulting amino acid substitution is G387P.

48. (Previously Presented) A kit comprising a first enzyme and a second enzyme, wherein said first enzyme is a Taq DNA polymerase, and said second enzyme is the wild type Pfu DNA polymerase comprising the amino acid sequence of SEQ ID NO. 12, except that it is mutated at an amino acid selected from the group consisting of Y410, T542, D543, K593, Y595, Y385, G387 and G388, or combinations thereof, and packaging material therefor, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity as compared to the wild type Pfu DNA polymerase.



49. (Previously Presented) A kit comprising a first enzyme and a second enzyme, wherein said first enzyme is a KOD DNA polymerase, and said second enzyme is the wild type Pfu DNA polymerase comprising the amino acid sequence of SEQ ID NO. 12, except that it is mutated at an amino acid selected from the group consisting of D405, Y410, T542, D543, K593, Y595, Y385, G387 and G388, or combinations thereof, and packaging material therefor, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity as compared to the wild type Pfu DNA polymerase.

50. (Previously Presented) A kit comprising a first enzyme and a second enzyme, wherein said first enzyme is a JDF-3 DNA polymerase having the sequence shown in SEQ ID NO. 10, and said second enzyme is the wild type Pfu DNA polymerase comprising the amino acid sequence of SEQ ID NO. 12, except that it is mutated at an amino acid selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387 and G388, or combinations thereof, and packaging material therefor, and wherein said second enzyme possesses 3'-5' exonuclease activity and reduced 5'-3' DNA polymerization activity as compared to the wild type Pfu DNA polymerase.

51. (Previously Presented) The kit of claim 48, 49, or 50, wherein said kit further comprises a reagent selected from the group consisting of: dNTPs, reaction buffer, primer, and DNA enhancing factor.